



# The Open Microbiology Journal Supplementary Material

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## Production and Characterization of L-Asparaginases of *Streptomyces* Isolated from the Arauca Riverbank (Colombia)

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Article History	Received: January 16, 2019	Revised: May 16, 2019	Accepted: May 22, 2019
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### SUPPLEMENTARY EQUATIONS, FIGURES AND TABLES

Logistic equation.

$$\frac{dX}{dt} = \mu_m X \left(1 - \frac{X}{X_m}\right) \quad (\text{S1})$$

Integrated form of the logistic equation considering  $X=X_0$  at  $t=0$ .

$$X = \frac{X_0 e^{\mu_m t}}{1 - \left(\frac{X_0}{X_m}\right)(1 - e^{\mu_m t})} \quad (\text{S2})$$

$$\ln \frac{X}{X_m - X} = \mu_m t - \ln \left(\frac{X_m}{X_0} - 1\right) \quad (\text{S3})$$

Luedeking-Piuret model

$$\frac{dP}{dt} = m \frac{dX}{dt} + nX \quad (\text{S4})$$

Integrated form of the Luedeking-Piuret model with  $P=0$  at  $t=0$

$$P = m X_0 \left( \frac{e^{\mu_m t}}{\left(1 - \frac{X_0}{X_m}\right)(1 - e^{\mu_m t})} - 1 \right) + n \frac{X_m}{\mu_m} \ln \left( 1 - \frac{X_0}{X_m} (1 - e^{\mu_m t}) \right) \quad (\text{S5})$$

Equation 5 in a simple form

$$P = m \alpha(t) + n \beta(t) \quad (\text{S6})$$

When it is assumed that  $\{dX / dt = 0\}$  and  $\{X = X_m\}$  in the Luedeking-Piuret model; the next expression can be used to determine the value 'n'.

$$n = \frac{\left(\frac{dP}{dt}\right)_{\text{stationary phase}}}{X_m} \quad (\text{S7})$$

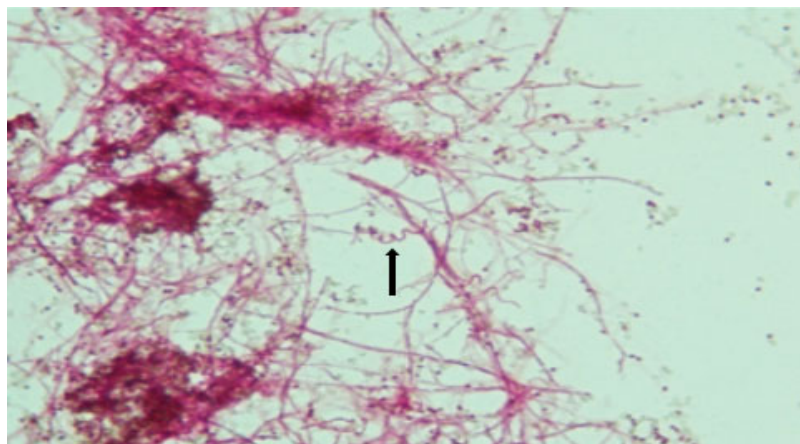


Fig. (S1). Gram stain of *Streptomyces lacticiproducens* grown in ISP-5 liquid medium for 7 days at 30°C. Observed in the 100x objective.

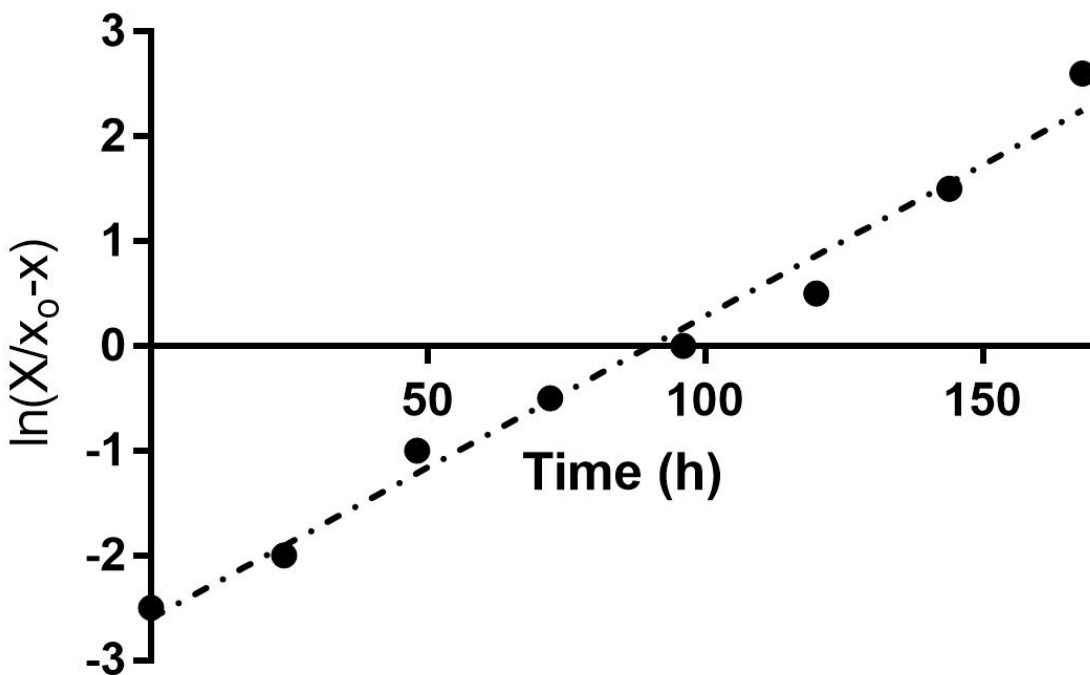


Fig. (S2). Graphical determination of kinetic parameters  $\mu_m$  and  $X_m$ .

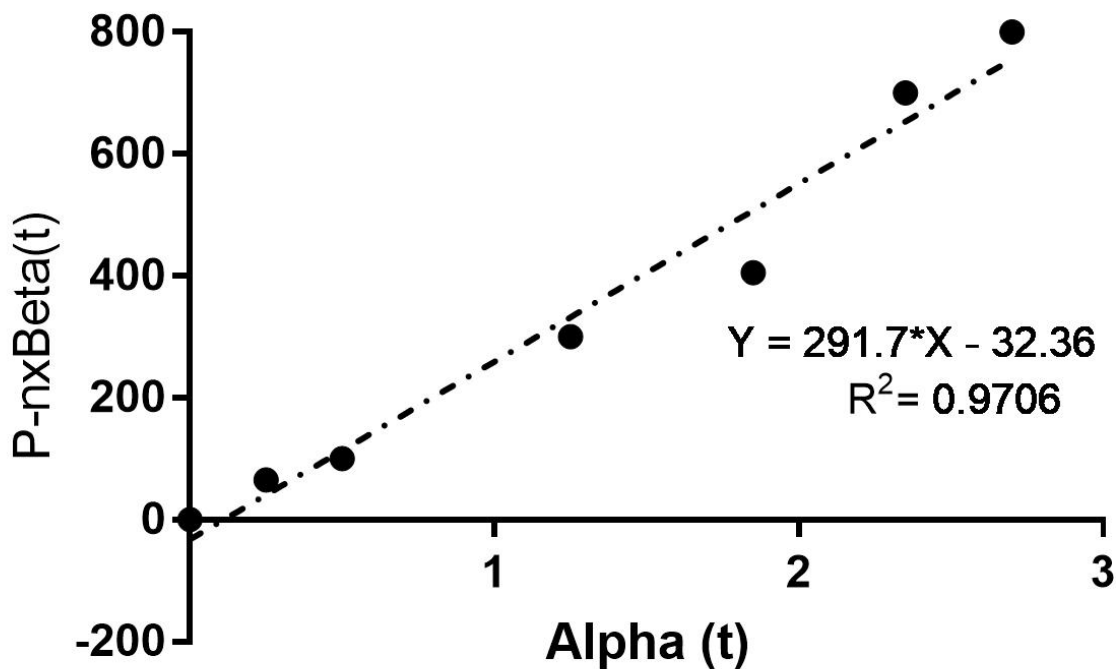


Fig. (S3). Graphical determination of constants associated with no growth ( $m$ ) and growth ( $n$ ) of the L-asparaginase production.

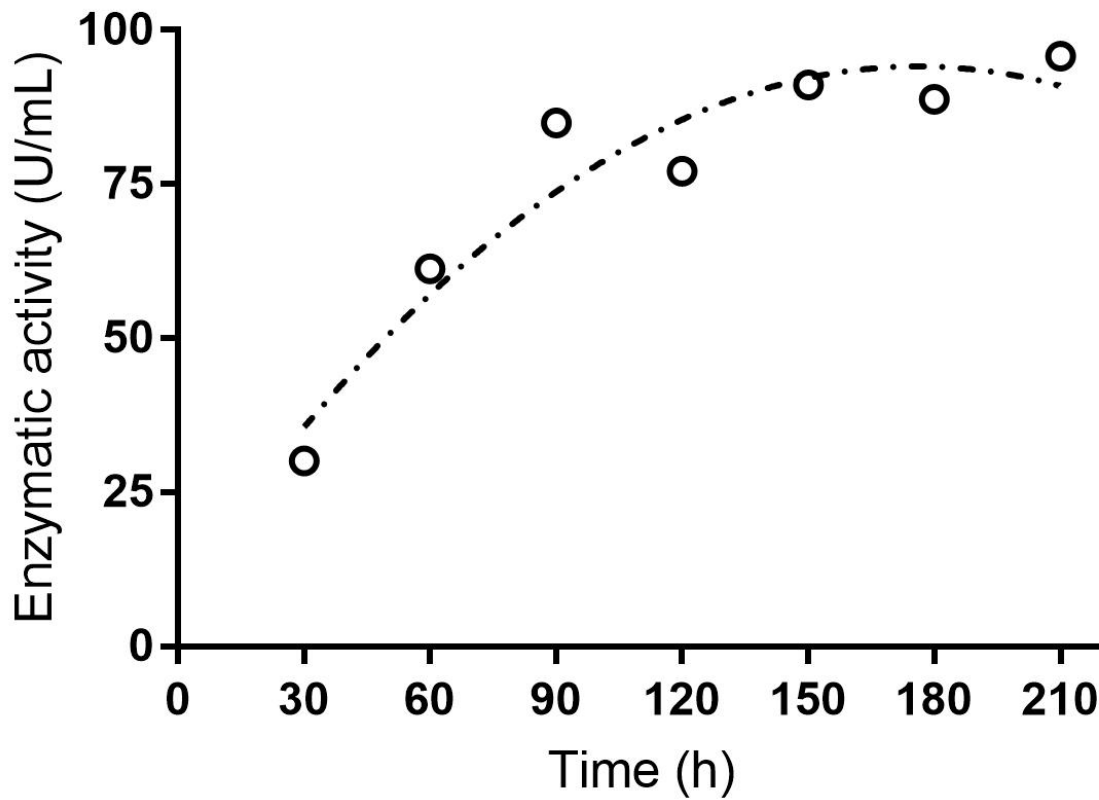


Fig. (S4). Comparison of the experimental data (o) and the Luedeking-Piret model (---) in the production of L-asparaginase by *S. lacticiproducens*.

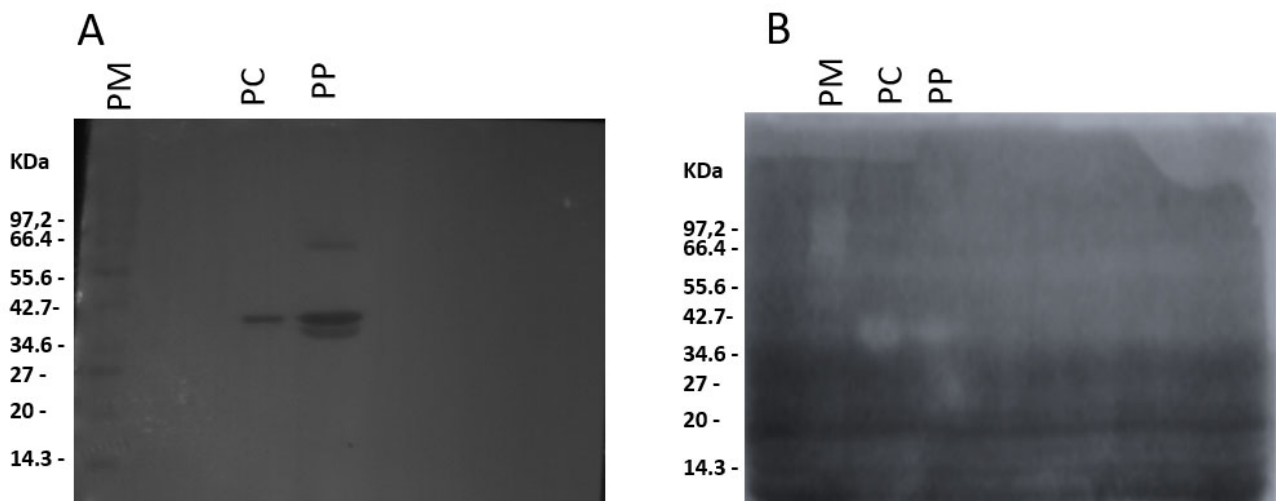


Fig. (S5). A. Western Blot of purified of L-asparaginase produced by *S. lacticiproducens* with L-asparaginase antibody. PM, Protein marker. PC, Positive control. PP, Purified protein 112. B. Silver staining of purified L-asparaginase produced by *S. lacticiproducens* with L-asparaginase antibody. PM, Protein marker. PC, Positive control. PP, Purified protein 112.

**Table S1. Definition of the variables and levels in the design of Plackett-Burman for the activity L-asparaginase for the selected *Streptomyces* strains.**

Run	Variables				
	Carbon source	Nitrogen source	pH	Temperature	Agitation rate
1	1	-1	-1	1	1
2	-1	-1	1	-1	1
3	1	-1	1	1	-1
4	-1	1	-1	1	-1
5	-1	-1	-1	1	-1
6	1	-1	1	-1	1
7	1	1	1	-1	-1
8	1	1	-1	-1	1
9	1	-1	-1	-1	-1
10	1	1	-1	-1	-1
11	-1	-1	1	1	-1
12	-1	1	1	-1	-1
13	-1	1	1	1	1
14	-1	-1	-1	-1	1
15	-1	1	-1	1	1
16	1	1	-1	1	1
17	1	-1	1	1	1
18	-1	-1	-1	-1	-1
19	-1	1	1	-1	1
20	1	1	1	1	-1
Level	g/L	g/L	pH	°C	rpm
-1	1.0	0.1	6.0	30.0	100
1	1.5	0.2	8.0	35.0	200

**Table S2. Taguchi L9 Orthogonal Array for the experimental design in the activity L-asparaginase for the selected *Streptomyces* strains.**

Run	Coding of the levels of independent variables	
	Concentration of carbon source	Concentration of nitrogen source
1	1	1
2	1	2
3	1	3
4	2	1
5	2	2
6	2	3
7	3	1
8	3	2
9	3	3
Level	g/L	g/L
1.0	1.5	0.20
2.0	2.0	0.25
3.0	2.5	0.30

Table S3. Culture characteristics and molecular identification of the *Streptomyces* selected.

	MEDIUM/STRAIN	5	67	112	126	220	276	326
ISP2	Aerial mycelium	Gray-White	Gray-Blue	White-Yellow	White-Yellow	No color	White-Yellow	Gray-White
	Vegetative mycelium	White	White	White-Yellow	Yellow	White	Yellow	White
	Diffusible pigment	No Change	No Change	No Change	Yellow	White	Yellow	White
	Growth	+++	+++	++	+++	++	+++	+++
ISP3	Aerial mycelium	Gray-White	Red-White	White-Yellow	Gray	White-Brown	Brown	Gray-White
	Vegetative mycelium	White	Red	Brown	Yellow	Brown	Yellow	Not shown
	Diffusible pigment	White	Red	Brown	Yellow	Brown	Yellow	No Change
	Growth	+++	+++	+++	+++	+++	+++	+++
ISP4	Aerial mycelium	White	White	White-Brown	White	White	White-Brown	White
	Vegetative mycelium	Not shown	Not shown	Not shown	Not shown	Not shown	Not shown	Not shown
	Diffusible pigment	No Change	No Change	No Change	No Change	No Change	No Change	No Change
	Growth	++	++	++	++	++	++	++
ISP5	Aerial mycelium	White-Black	Red-Brown	White	White-Black	White-Black	Yellow	White
	Vegetative mycelium	White	Red-Orange	White	White	White	White	White
	Diffusible pigment	No Change	No Change	No Change	No Change	Yellow	Yellow	Yellow
	Growth	++	++	++	++	++	++	++

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